

Adipokinetic Neuropeptides and Flight Metabolism in Three Moth Species of the Families Sphingidae, Saturniidae and Bombycidae

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Methanolic extracts from corpora cardiaca of three moth species, *Hippoteon eson* (Sphingidae), *Imbrasia cytherea* (Saturniidae) and *Bombyx mori* (Bombycidae) show adipokinetic activity in conspecific bioassays. Haemolymph carbohydrates in these moths are not affected. These extracts are also active in heterologous bioassays: haemolymph lipids are increased in *Locusta migratoria*, whereas a small effect on haemolymph carbohydrates was observed in *Periplaneta americana*. Therefore, locusts can be used to monitor adipokinetic activity in corpora cardiaca from moth extracts during isolation. The three moth species possess an adipokinetic peptide with the same retention time on reversed phase high performance liquid chromatography (RP-HPLC) as a peptide isolated previously from *Manduca sexta*, which was code-named Mas-AKH. *H. eson* contains a second active peak with a similar retention time on RP-HPLC as the hypertrehalosaemic peptide isolated previously from *Helicoverpa zea*, code-named Hez-HrTH. Both synthetic peptides, Mas-AKH and Hez-HrTH, produce an adipokinetic effect in the three experimental moth species. In *H. eson*, the haemolymph concentration of Mas-AKH or Hez-HrTH needed to elicit a maximum hyperlipaemic response is about 20 to 30 nm.

Flight behaviour in the three moth species is quite different: *H. eson* is a good hovering flyer, *I. cytherea* is a comparatively bad flyer and *B. mori* males show only degenerate flight movements during their mating dance. Haemolymph lipid levels in *H. eson* decrease drastically during 15 min of flight and return to pre-flight levels in a subsequent rest period. The amount of lipids metabolized during flight is 10.9 mg/g×hr. Haemolymph carbohydrate levels drop during flight, but remain low during the 45 min of recovery. Haemolymph lipids in “dancing” males of *B. mori* remain constant. In individuals, however, which have low initial lipid levels in the blood, lipid concentrations increase significantly in a subsequent 15 min rest period after “dancing”. Metabolic changes during flight in *I. cytherea* were not investigated due to this species’ poor flight performance.

Introduction

Adipokinetic and hypertrehalosaemic peptides have been isolated from corpora cardiaca of almost all major insect orders (Gäde, 1990a). The peptides are grouped into the so-called adipokinetic hormone/ red pigment-concentrating hormone family (AKH/RPCH family). Members of this family are octa-, nona- or decapeptides with blocked termini (5-oxopyrrolidine-2-carboxylic acid at the N-terminus; the C-terminus is amidated).

Two AKH/RPCH peptides have been isolated from Lepidopteran species so far: The nonapeptide Mas-AKH was found in *Manduca sexta*

(Sphingidae; Ziegler *et al.*, 1985), *Helicoverpa [Heliothis] zea* (Noctuidae; Jaffe *et al.*, 1986), *Pseudaletia unipuncta* (Noctuidae; Orchard *et al.*, 1991) and in *Bombyx mori* (Bombycidae; Ishibashi *et al.*, 1992). The decapeptide Hez-HrTH has been isolated and sequenced from *H. zea* (Jaffe *et al.*, 1988).

AKH/RPCH peptides ensure the availability of metabolites (mainly lipids and carbohydrates, but possibly also amino acids) during energy demanding processes such as the flight of insects (Gäde, 1992a). Previous studies have shown that in continuous flyers like *P. unipuncta*, lipid levels increase steadily during flight (Orchard *et al.*, 1991), whereas during the hovering tethered flight of *M. sexta* haemolymph lipids and carbohydrates decrease (Ziegler and Schulz, 1986a,b). In the latter species, haemolymph lipids return to pre-flight levels during a resting period after flight.

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In the present study, three moth species with very different flying abilities were compared: *Hippoteon eson* (Sphingidae) is a good hovering flyer (Jarvis, 1987), *Imbrasia [Nudaurelia] cytherea* (Saturniidae) is a relatively bad, irregular flyer (Geertsma, 1975) and *Bombyx mori* males (Bombycidae) show only rather degenerate flight movements during their mating dance (Kanzaki *et al.*, 1992). In a first step the presence of AKH/RPCH-like neuropeptides and their effect on haemolymph metabolites (lipids and carbohydrates) of resting moths was determined. In a second step, levels of these metabolites were monitored before and after flight, as well as after a recovery period.

Materials and Methods

Insects

For heterologous bioassays (see below) 15- to 25-day-old male migratory locusts (*Locusta migratoria*) and adult male cockroaches (*Periplaneta americana*) were used, which were reared as described previously (Gäde, 1991; Gäde, 1992b).

Different larval stages of the common striped hawk moth (*Hippoteon eson*, Sphingidae) were caught in parks around Cape Town during the months April to June and November/ December. The larvae were transferred to glass cages and kept at room temperature (22 °C) under natural light conditions. They were fed daily with fresh arum lily (*Zantedeschia aethiopica*) leaves. Pupae were kept at the same temperature and light conditions, and adult moths emerged after one month. These were used on the day of imaginal moult for bioassays, flight experiments or corpora cardiaca preparations.

Adult pine tree emperor moths (*Imbrasia [Nudaurelia] cytherea*, Saturniidae) were caught in a pine forest near Grabouw (Cape Province) during the months April/ May. Corpora cardiaca were dissected on the same day.

Silk moth larvae (*Bombyx mori*, Bombycidae) were reared during the months September to January at room temperature and natural light conditions. They were fed daily with fresh mulberry leaves. Adult moths were sacrificed on the day of imaginal moult or used for bioassays and activity experiments.

Bioassays

Haemolymph sampling, hyperlipaemic and hypertrehalosaemic bioassays in *L. migratoria* and *P. americana*, respectively, were as in Gäde (1980).

I. cytherea: Males were used on the day of capture. They were allowed to settle for several hours in a large cage. During bioassays the moths were kept in groups of eight in smaller cages (20 l volume). Haemolymph samples were obtained by puncturing the dorsal vessel after the scales were removed. The moths were injected laterally into the abdomen as described above for *L. migratoria/P. americana*.

H. eson: Males and females on the day of imaginal moult were used. Haemolymph sampling and injections were as described for *I. cytherea*. During the experiment the animals were kept isolated under funnels to prevent activity.

B. mori: Males were used on the day of imaginal moult. Conditions were the same as described for *H. eson*, except that haemolymph samples were obtained by pricking the intersegmental membrane of the abdomen dorsally.

All haemolymph samples were taken at the beginning of the experiment and 90 minutes (lipids) or 120 minutes (carbohydrates) after injections. Lipid and carbohydrate levels were determined as in *L. migratoria/P. americana* (Gäde, 1991; Gäde, 1992b).

Preparation of corpora cardiaca and head extracts; further purification

Corpora cardiaca were dissected on the day of imaginal moult from *H. eson* and on the day of capture from *I. cytherea*. Peptides were extracted with methanol according to Gäde *et al.* (1984). It was not possible to reliably dissect the corpora cardiaca from *B. mori*. Therefore, head segments excluding the eyes and the antennae but including the brain and corpora cardiaca were cut with a sharp blade and transferred to 80% methanol, sonicated and centrifuged at 6000×g for 10 min. The resulting pellet was re-extracted twice with 80% methanol and re-centrifuged. The combined supernatants were dried by vacuum centrifugation and dissolved in distilled water for further use in the bioassays. For further purification the aqueous sample was loaded on a Sep-Pak C-18 RP car-

tridge (Millipore Corporation, Milford, Massachusetts, U.S.A.). This was washed with 18%, 60% and 100% acetonitrile containing 0.1% trifluoroacetic acid (TFA). The 60% fraction, which in pilot experiments proved to contain Mas-AKH and Hez-HrTH, was dried by vacuum centrifugation and used for RP-HPLC.

Methanolic extracts of corpora cardiaca or of heads were subjected to RP-HPLC according to Gäde (1985a). For further details on HPLC-conditions see legends to figures 1A to 1C.

Flight experiments

Before commencement of flight experiments, moths were kept separately under funnels (*H. eson*) or in beakers (*B. mori*), for at least two hours in order to ensure basal metabolism.

H. eson: A thread was glued onto the shaved thorax. Animals were allowed to perform a continuous hovering flight for 15 minutes. Haemolymph samples were taken before and immediately after the flight as well as after 15 and

Table I. Effects on haemolymph lipids and carbohydrates respectively upon injection of moth corpora cardiaca or head extracts into acceptor locusts and cockroaches.

		Water	<i>B. mori</i> 0.5 Head equivalents	10 pmol Lom-AKH-I/ Pea-CAH-I**
<i>L. migratoria</i> Haemolymph Lipids	0 min	15.7 ± 3.1*	15.7 ± 6.0	18.2 ± 4.8
	90 min	18.4 ± 5.2	38.1 ± 12.1	85.5 ± 16.3
	difference	2.7 ± 2.5	22.4 ± 6.9 ^a	67.3 ± 14.5 ^a
	<i>n</i>	13	12	12
<i>P. americana</i> Haemolymph Carbohydrates	0 min	18.3 ± 1.3	17.1 ± 3.2	18.5 ± 2.3
	120 min	21.4 ± 2.3	22.4 ± 5.4	39.0 ± 6.0
	difference	3.1 ± 1.7	5.3 ± 3.3 ^c	20.5 ± 5.1 ^a
	<i>n</i>	7	7	7
		Water	<i>I. cytherea</i> 1 CC equivalent	10 pmol Lom-AKH-I/ Pea-CAH-I
<i>L. migratoria</i> Haemolymph Lipids	0 min	9.5 ± 3.5	8.6 ± 2.4	9.2 ± 2.4
	90 min	11.2 ± 4.0	20.8 ± 6.8	52.9 ± 15.9
	difference	1.7 ± 1.8	12.2 ± 3.1 ^a	43.7 ± 14.3 ^a
	<i>n</i>	14	7	15
<i>P. americana</i> Haemolymph Carbohydrates	0 min	15.0 ± 2.9	16.3 ± 3.2	17.8 ± 3.1
	120 min	17.1 ± 5.3	21.8 ± 3.7	41.6 ± 11.6
	difference	2.1 ± 3.3	5.5 ± 1.5 ^a	23.8 ± 9.0 ^a
	<i>n</i>	12	12	12
		Water	<i>H. eson</i> 1 CC equivalent	10 pmol Lom-AKH-I/ Pea-CAH-I
<i>L. migratoria</i> Haemolymph Lipids	0 min	10.7 ± 4.0	10.8 ± 3.3	10.8 ± 2.4
	90 min	9.7 ± 2.8	40.1 ± 9.9	52.4 ± 17.2
	difference	-1.0 ± 1.3	29.3 ± 8.1 ^a	41.6 ± 15.1 ^a
	<i>n</i>	6	8	8
<i>P. americana</i> Haemolymph Carbohydrates	0 min	15.8 ± 2.5	14.0 ± 4.1	16.4 ± 1.6
	120 min	17.0 ± 2.8	21.7 ± 4.1	33.6 ± 5.4
	difference	1.2 ± 0.9	7.7 ± 3.4 ^a	17.2 ± 4.9 ^a
	<i>n</i>	8	8	8

* Values are given in mg/ml as means ± standard deviation. Significance of changes in haemolymph metabolites grouped in probability levels: ^a ($p \geq 0.99$); ^b ($0.95 < p < 0.99$) and ^c ($p \leq 0.95$).

** Maximum responses in acceptor insects: injection of 10 pmol Lom-AKH-I in locusts or 10 pmol Pea-CAH-I in cockroaches, respectively.

45 minutes of rest after flight. During rest the moths were kept under funnels (cf. bioassays above). Control animals were kept under funnels all the time and haemolymph was taken according to the same time regime.

B. mori: Males and females were placed into a small box, but sexes were separated by meshed wire. Under these conditions males perform a "mating dance" including continuous flapping movements with their wings. Haemolymph was taken according to the same time regime as given for *H. eson*. Resting and control animals were kept isolated in closed beakers.

Determination of haemolymph volume

The haemolymph volume of adult *H. eson* was determined according to the method of Clegg and Evans (1961). In brief, a trace amount of tritium labelled inulin dissolved in 10 µl of water (equal to 145000 cpm) was injected into the moths as described above. After 20 min of incubation an aliquot of haemolymph was taken, pipetted into a scintillation vial containing 4 ml of scintillation fluid (Packard, Ultima Gold TR) and the radioactivity was measured in a Tri Carb 460 instrument (Packard).

Statistical procedures

A two sample analysis determining the confidence interval for the difference of means and an analysis of variance (ANOVA) was performed. Probability levels were grouped in $\geq 99\%$ (a), 95–99% (b) and $\leq 95\%$ (c).

Synthetic peptides

The synthetic peptides from locusts (Lom-AKH-I), cockroaches (Pea-CAH-I) and moths (Mas-AKH and Hez-HrTH) came from Peninsula Laboratories (Belmont, CA, U.S.A.).

Results

Presence of biological activity in moth corpora cardiaca and head extracts

Table I shows the effects of crude CC or head extracts upon injection into acceptor locusts and cockroaches. The small amounts of extract injected caused in all cases only sub-maximum responses, but they revealed differences in the two acceptor systems. The extracts from all three moth species caused a substantial hyperlipaemic response in locusts, ranging from 31% to 70% of the possible maximum response. The same amount of extract elicited only a poor hypertrehalosaemic effect in *P. americana*, reaching 23% to 39% of the possible maximum response. Consequently, the hyperlipaemic locust bioassay was used as a routine monitoring procedure of biological activity in moth CC- and head extracts during the isolation procedures.

In the next step, extracts of the three moth species were separated by RP-HPLC and fractions were tested for biological activity (see above). In *B. mori* and *I. cytherea* only one fraction was biologically active which, in the case of *I. cytherea*, corresponded with an UV-peak showing the same retention time as synthetic Mas-AKH (Fig. 1A).

Table II. Conspecific injections of corpora cardiaca or head extracts and their effect on haemolymph lipids and carbohydrates.

		<i>B. mori</i>		<i>I. cytherea</i>		<i>H. eson</i>	
		Water	1.7 Head equivalents	Water	1 CC equivalent	Water	1 CC equivalent
Haemolymph Lipids	0 min	27.4 ± 12.9*	25.0 ± 14.3	82.7 ± 13.2	75.7 ± 11.2	30.3 ± 11.8	29.5 ± 11.8
	90 min	26.5 ± 12.1	44.4 ± 18.9	86.0 ± 15.3	90.3 ± 16.5	34.3 ± 12.5	51.4 ± 17.7
	difference	-0.9 ± 2.6	19.4 ± 6.7 ^a	3.3 ± 7.6	14.6 ± 8.2 ^a	4.0 ± 4.6	21.9 ± 12.5 ^a
	n	14	8	12	11	9	8
Haemolymph Carbohydrates	0 min	9.1 ± 2.4	7.0 ± 0.9	5.3 ± 2.6	8.1 ± 1.8	23.0 ± 3.0	21.4 ± 4.8
	120 min	8.0 ± 3.5	6.4 ± 1.0	5.9 ± 0.7	7.9 ± 1.2	22.1 ± 3.2	23.2 ± 4.8
	difference	-1.1 ± 3.7	-0.6 ± 0.7 ^c	0.6 ± 2.2	-0.2 ± 1.2 ^c	-0.9 ± 2.6	1.8 ± 2.2 ^c
	n	7	6	5	7	8	8

* Values are given in mg/ml as means ± standard deviation. Significance levels of changes in haemolymph metabolites are grouped in ^a ($p \geq 0.99$); ^b ($0.95 < p < 0.99$) and ^c ($p \leq 0.95$).

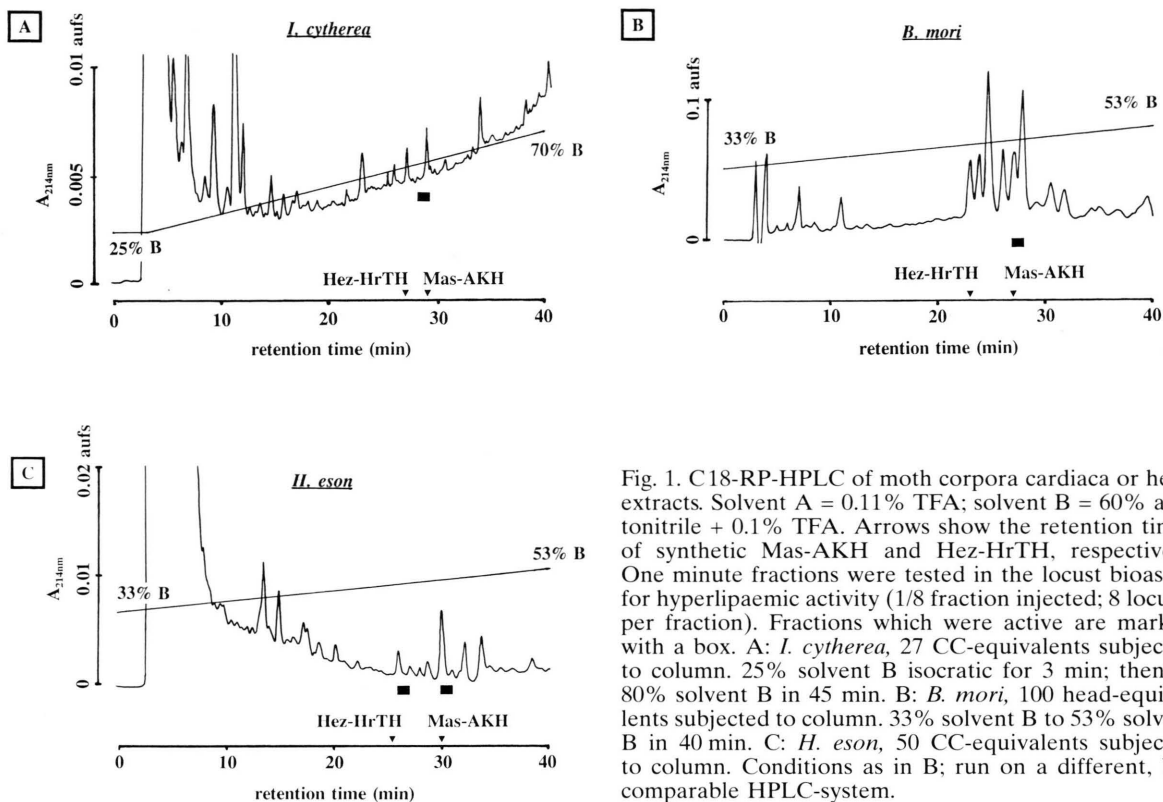


Fig. 1. C18-RP-HPLC of moth corpora cardiaca or head extracts. Solvent A = 0.11% TFA; solvent B = 60% acetonitrile + 0.1% TFA. Arrows show the retention times of synthetic Mas-AKH and Heiz-HrTH, respectively. One minute fractions were tested in the locust bioassay for hyperlipaemic activity (1/8 fraction injected; 8 locusts per fraction). Fractions which were active are marked with a box. A: *I. cytherea*, 27 CC-equivalents subjected to column. 25% solvent B isocratic for 3 min; then to 80% solvent B in 45 min. B: *B. mori*, 100 head-equivalents subjected to column. 33% solvent B to 53% solvent B in 40 min. C: *H. eson*, 50 CC-equivalents subjected to column. Conditions as in B; run on a different, but comparable HPLC-system.

The active peak from *B. mori* head extracts was also eluted at the same retention time as Mas-AKH, but no distinct peak was seen; it is assumed that the active peak was buried under other co-eluting material (Fig. 1B). In *H. eson* two fractions were active in bioassays and both were associated with distinct UV peaks (Fig. 1C). One peak co-eluted with synthetic Mas-AKH, whereas the other peak had a retention time of 26.1 min, which was slightly longer than the one of synthetic Heiz-HrTH (25.8 min).

Conspecific injection of the extracts was performed in non-ligated adult moths (Table II). Initial lipid concentrations, before injection, were comparable in *B. mori* and *H. eson* but were higher in *I. cytherea*. Initial carbohydrate concentrations were higher in *H. eson* than in the other two species. This results in a lipid to carbohydrate ratio of about 3 in *B. mori*, 16 in *I. cytherea* and 1 in *H. eson*. After injection of their own extracts we observed an increase of the haemolymph lipid concentrations in all three species. Haemolymph

carbohydrate levels, however, were not significantly affected.

Moreover, the synthetic peptides Mas-AKH and Heiz-HrTH were injected into the three moth species and their effect on haemolymph lipid levels was determined. In *B. mori* 6 pmol of Mas-AKH caused an increase in haemolymph lipid concentrations of 18.3 ± 9.6 mg/ml ($n = 6$). The increase was 13.9 ± 6.3 mg/ml ($n = 6$) with 10 pmol of Heiz-HrTH. In *I. cytherea* 12.5 pmol Mas-AKH elevated haemolymph lipid concentrations by 17.0 ± 10.5 mg/ml ($n = 12$). In *H. eson*, the increase was 19.9 ± 11.7 mg/ml ($n = 15$) with 4 pmol of Mas-AKH and 19.1 ± 8.2 mg/ml ($n = 13$) with 5.5 pmol Heiz-HrTH. Increases of haemolymph lipids in all species were significantly higher than those in water-injected controls ($p \geq 99\%$). In *B. mori* and in *H. eson*, higher concentrations of synthetic Mas-AKH (and head extracts in *B. mori*) were also tested, which did not lead to a further increase of haemolymph lipid concentrations (results not

shown). This indicates, that a maximum hyperlipaemic response was already reached.

Flight experiments

The hovering flight of *H. eson* and the "mating flight" of *B. mori* were investigated. No flight experiments were executed with *I. cytherea*. Although this species has been observed to fly in its natural habitat during dusk and dawn (Geertsma, 1975), this activity is not consistent and resulted only in gliding but not active flight (own observations). In the laboratory no continuous flight behaviour could be evoked (own observations). Males of *B. mori* which had higher lipid levels, performed better than those with lower lipid levels (≤ 30 mg/ml). Ligation paralysed the insects and was therefore not used for the controls.

Figure 2A to 2C shows the decrease/ increase of haemolymph lipid/ carbohydrate levels during flight. Lipid levels in *H. eson* decreased signifi-

cantly from 36.8 ± 7.2 mg/ml ($n = 8$) before flight to 20.7 ± 3.7 mg/ml after a 15 min flight. During a subsequent rest period of 15 min lipid levels reached the pre-flight level; after 45 min of rest after flight, lipids were even 20% higher than before flight (Fig. 2A). The concentration of total carbohydrates in the haemolymph dropped significantly to 58% of the pre-flight level of 18.6 ± 6.2 mg/ml, when insects were flown for 15 min. Even after 45 min of recovery, pre-flight levels were not reached again (Fig. 2B). In resting control animals lipid and carbohydrate levels remained more or less constant (Fig. 2A and 2B).

Concentrations of haemolymph lipids did not change significantly in *B. mori* after 15 minutes of mating dance (Fig. 2C). There was, however, a slight increase after a further 15 minute period of resting. This increase occurred in all animals tested. It was only significant, however, in males with initial values below 30 mg/ml (increase of about 6.6 mg/ml reaching 129% of the zero time

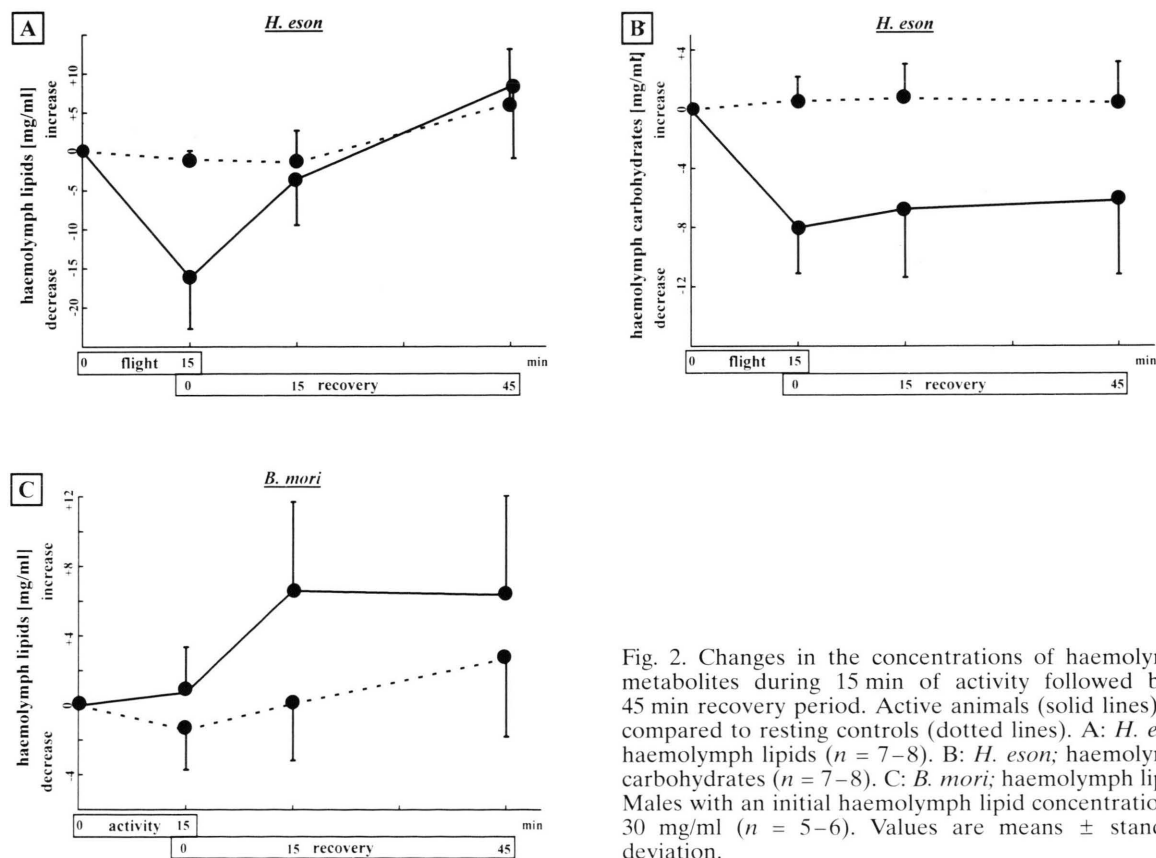


Fig. 2. Changes in the concentrations of haemolymph metabolites during 15 min of activity followed by a 45 min recovery period. Active animals (solid lines) are compared to resting controls (dotted lines). A: *H. eson*; haemolymph lipids ($n = 7-8$). B: *H. eson*; haemolymph carbohydrates ($n = 7-8$). C: *B. mori*; haemolymph lipids. Males with an initial haemolymph lipid concentration ≤ 30 mg/ml ($n = 5-6$). Values are means \pm standard deviation.

value of 22.5 ± 2.5 mg/ml; $n = 6$). 45 minutes after activity, lipid levels remained at this higher concentration. In resting controls, haemolymph lipids remained at a constant level (19.8 ± 6.8 mg/ml; $n = 5$); there was a slight, but not significant, increase at the end of the observation period.

Discussion

Presence of biological activity in moth corpora cardiaca/head extracts

Injection of crude extracts of corpora cardiaca have been investigated only in a few Lepidopteran species with no or only a very weak response in acceptor *L. migratoria* and *P. americana* (reviewed in Gäde, 1990a). In the present study *I. cytherea* and *H. eson* CC extracts and *B. mori* head extracts were tested. All of them elicited a hyperlipaemic response in *L. migratoria*, but were only poorly active in *P. americana*. Similar results were reported by Gäde (1990b) upon injection of synthetic Mas-AKH into migratory locusts and American cockroaches.

Conspecific injections of moth crude CC extracts have been investigated in a number of species and mostly caused a hyperlipaemic response (the numbers in brackets give the amount needed to elicit a maximum response): *M. sexta* (0.01 CC-equivalents; Ziegler and Schulz, 1986a), *Sphinx ligustri* (Gäde and Scheid, 1986), *Acherontia atropos* (Gäde, 1985b), *H. zea* (1 CC-equivalent; Jaffe *et al.*, 1986), *B. mori* (3 head equivalents; Ishibashi *et al.*, 1992) and *P. unipuncta* (0.6 CC-equivalents; Orchard *et al.*, 1991). Conspecific injections also resulted in hyperlipaemia in the three moth species of the present study. Injections in *B. mori* were performed in non-ligated animals, in contrast to the experiments by Ishibashi *et al.* (1992). Although initial haemolymph lipid levels for *B. mori* were higher in the present study, the maximum increase of lipids upon injection of 1.7 head equivalents was in the same range as given by Ishibashi *et al.* (1992). Injection of 1 CC-equivalent caused a maximum hyperlipaemic response in *H. eson*. Control haemolymph lipid and carbohydrate levels were in the same range as in *M. sexta* (Ziegler, 1991) but the maximum increase of haemolymph lipids (Ziegler, 1990) was considerably lower in *H. eson*. Like in *B. mori* and *I. cytherea* no effect on haemolymph carbohydrates could

be seen in *H. eson* when injected conspecifically. Up to now a hyperglycaemic effect in moths was only reported for *H. zea* (Jaffe *et al.*, 1988).

Injection of the synthetic moth hormones Mas-AKH and Hez-HrTH also caused a hyperlipaemic response in the three species of the present study. In *H. eson*, a maximum hyperlipaemic response was reached with about 4 pmol Mas-AKH or 5.5 pmol Hez-HrTH per animal. This compares well with the values given for other moths: 2 to 10 pmol in *M. sexta* (Fox and Reynolds, 1991; Ziegler, 1990) and 5 pmol in *P. unipuncta* (Orchard *et al.*, 1991). Given a haemolymph volume of 192.8 μ l, the adipokinetic hormone concentration needed to elicit a maximum hyperlipaemic response in *H. eson* is between 20 to 30 nM.

Using RP-HPLC, only one active peak could be found in *B. mori* (Fig. 1B), which has been identified previously as Mas-AKH (Ishibashi *et al.*, 1992). In *I. cytherea*, we also found only one biologically active peak. It had the same retention time as synthetic Mas-AKH (Fig. 1A). In *H. eson*, two active peaks were found, one of which co-eluted with synthetic Mas-AKH. The other peak showed slightly more hydrophobic behaviour than Hez-HrTH, which was run separately but under the same conditions. It is not possible to decide from these data whether the CC-extract of *H. eson* does contain a second adipokinetic peptide different in structure from Hez-HrTH. The very small difference in retention times between an activity peak of a crude extract and of a pure synthetic peptide may easily be explained by the higher amount of contaminating material subjected to the analytical column when a crude extract is isolated; such a factor may influence retention time on a reversed phase column.

Summarising our results so far it is clear that the data on bioactivity in combination with retention times in HPLC strongly suggest that all investigated species contain Mas-AKH. Additionally, *H. eson* CC-extracts certainly have a second activity peak which is possibly identical with Hez-HrTH; structure identification is necessary to answer this question.

Lipid and carbohydrate metabolism during flight

In order to further investigate the possible effect of peptide hormones on metabolites, levels of

lipids and carbohydrates were monitored during flight. A good hovering flyer (*H. eson*) and a bad, "degenerated" flyer (*B. mori*) were compared.

Changes of haemolymph lipids and carbohydrate levels during flight in *H. eson* (Fig. 2A and 2B) resembled the pattern found in *M. sexta* (Ziegler and Schulz, 1986a,b). *M. sexta* haemolymph lipids decrease during flight. While flying, the mobilization of lipids is increased until the rate of mobilization is equal to that of utilization and after 30 min of flight a steady state is reached. This mobilization of lipids from the fat body is controlled by Mas-AKH. Evidence for this is given by cardioectomy experiments, where haemolymph lipids drop during flight and remain low in a subsequent recovery period (Ziegler and Schulz, 1986a). The same authors suggest that the decrease of haemolymph lipids in the first minutes of flight can be used to estimate rates of oxidation for lipids used in flight. This can only be a rough calculation, because at the same time lipids are mobilized from the fat body and other fuels are possibly used as well. The estimate for *M. sexta* is 18 ml O₂/hr×animal or 11–13 ml O₂/hr×g (Ziegler and Schulz, 1986a). The sphingids *M. sexta* and *H. eson* have nearly the same mass (1.14 g versus 1.4–1.6 g; this study; Ziegler and Schulz, 1986a), and therefore possibly comparable energy requirements and oxygen consumption rates during flight occur. In *H. eson*, the decrease in haemolymph lipids was 16.1 mg/ml during 15 min of flight, which is comparable to the 15 min value given for *M. sexta* (Ziegler and Schulz, 1986a). Given a known haemolymph volume of 192.8 µl, the decrease in haemolymph lipids was 12.4 mg/ hr×animal or 10.9 mg/g×hr and the subsequent increase in haemolymph lipids during rest after flight is in the same range. Estimates of oxygen consumption for the oxidization of lipids can be based on tristearylglycerol-oxidation ($C_{57}H_{110}O_6 + 81.5 O_2 \rightarrow 57 CO_2 + 55 H_2O$), which requires 2.05 l O₂/g to be fully oxydized. Using this value an oxygen consumption rate of 25 ml O₂/hr×animal or 22 ml O₂/hr×g can be calculated for *H. eson*, which is twice as high as the data reported from *M. sexta* (see above). One possible reason for this difference may be that we used the value for 15 min of flight for our calculations in *H. eson*, whereas the 30 min value was used for *M. sexta*. Thus, in *H. eson*

steady state conditions may not have been reached (see above for *M. sexta*).

Like in *M. sexta* (Ziegler and Schulz, 1986b) carbohydrates could be used in *H. eson* at the onset of flight. In both species no significant mobilization from glycogen stores of the fat body occurs, otherwise haemolymph carbohydrate concentrations would increase in a rest period following flight. *H. eson* showed a decrease in haemolymph carbohydrates of 8.0 mg/ml during the first 15 min of flight which equals 1.5 mg carbohydrate, given the above haemolymph volume. The value reported for *M. sexta* is 1.8 mg carbohydrate during the first 5 min of flight (Ziegler and Schulz, 1986b). The main insect haemolymph sugar is trehalose (Gäde, 1991). Full oxidation of 1.5 mg trehalose in oxidative metabolism during 15 min of flight requires 4 ml O₂/hr×g, assuming 12 mol O₂/mol trehalose ($nO_2 + C_n(H_2O)_n \rightarrow nCO_2 + nH_2O$; in trehalose $n = 12$). This value is rather small, compared to the estimates for lipids given above and, therefore, carbohydrates contribute only negligible to energy provision during flight.

Based on the above data, *H. eson* can be classified as a lipid flyer, because lipids are its main fuel for flight. This resembles the situation in *M. sexta* (Ziegler and Schulz, 1986a). *H. eson* may possess two different adipokinetic hormones unlike *M. sexta*. It is possible that *H. eson* uses both adipokinetic hormones for lipid mobilization during flight like many other insects species, as reviewed by Gäde (1990a). However, this still has to be shown for *H. eson*.

The data of "mating flight" in *B. mori* were in sharp contrast to those in *H. eson*. Lipid levels in the blood did not change during activity, but in a subsequent rest period a slight, but significant, increase could be measured (Fig. 3C). During their "mating flight" *B. mori* males walk, fluttering their wings, but their bodies are too heavy to lift off (Kanzaki *et al.*, 1992). This activity is clearly not as energy demanding as the flight observed in *H. eson*. The most obvious interpretation of our data for *B. mori* is, that the use of haemolymph lipids during activity and their mobilization from the fat body by Mas-AKH could just be in balance (hence no change in lipid levels during activity). During a subsequent recovery period, however, mobilization from the fat body still takes place, but the lipids are not used because of lack of activ-

ity. This would explain the slight increase of haemolymph lipids after activity. In addition, this lipid mobilization is masked by a steady activity-unrelated, but CC- (Mas-AKH-) dependent increase of lipid levels as described by Ishibashi *et al.* (1992): Haemolymph lipids increase steadily in unligated males but remain constant in ligated males. Interestingly, an activity-unrelated, but CC- (Mas-AKH-) independent, increase of haemolymph lipids in conjunction with a decrease in haemolymph carbohydrates is reported for starving, adult *M. sexta* (Ziegler, 1991). Further investigations upon the exact role of Mas-AKH in *B. mori* and other species which do not show a high activity/ flight level, are necessary.

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